

Serial No. 10/724,233  
Atty. Docket No.: P63882US1

**Listing of Claims:**

1-83 (canceled).

84. (Previously presented) A method of solid phase peptide synthesis for preparing a ligand presenting assembly (LPA) for presentation of at least two identical peptide sequences having between 4 and 20 amino acids and having free C-terminal groups comprising the steps of:

a) assembling a plurality of identical, fully side-chain protected peptide sequences on a single solid phase resin support to provide a compound having the following formula:



wherein S represents the solid phase resin support, A represents a peptide sequence having between 4 and 20 naturally occurring L-amino acid residues, and a is  $\geq 2$ , and represents the number of fully side-chain protected peptide sequences on the resin support;

b) deprotecting any protected N-terminal amino groups while the peptide sequences are still attached to the resin support;

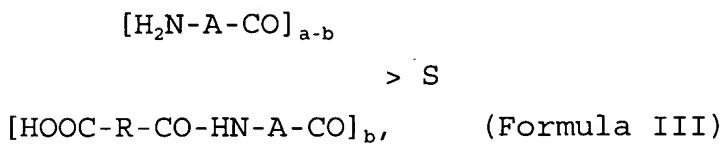
c) reacting the resulting compound having unprotected N-terminal amino groups in the peptide sequences with between 0.4 and 0.6 equivalents of an achiral dicarboxylic acid selected from the group consisting of: imino diacetic acid, 2-amino malonic acid, malonic acid, 3-amino glutaric acid and glutaric acid, being

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Fmoc, Boc or Aloc-protected on the amino or imino group, if present, thus having the following formula:



wherein R represents a  $N(X)(CH_2-)_2$ ,  $NH(X)CH<$ ,  $CH_2<$ ,  $NH(X)CH(CH_2-)_2$  or  $CH_2(CH_2-)_2$  group, and X represents an Fmoc, Boc or Aloc group, so that between 0.4 and 0.6 equivalents of said achiral dicarboxylic acid are added for every 1 equivalent of unprotected N-terminal amino group resulting in a compound with the following formula:



wherein b is between about 0.4a and 0.6a;

d) activating the product of step (c) (Formula III) so that the free carboxylic acid group reacts with the free N-terminal amino group; resulting in a compound of the following formula:

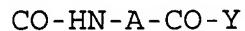


and

e) optionally splitting of any N-terminal Fmoc-group, Boc-group or Aloc-group;

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f) cleaving the product of step (e) from the resin support resulting in a LPA peptide sequence having the following formula:



R<

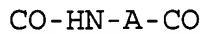


wherein, if N is present in R, X represents H, an Fmoc, Boc or Aloc group, and Y is OH or NH<sub>2</sub>.

85. (Previously presented) A method according to claim 84 further comprising the steps of prior to step (f)

(e') splitting of any N-terminal Fmoc, Boc or Aloc group originating from the dicarboxylic acid used in step (c) and

(e'') continuing the solid phase synthesis so as to provide a compound of the following formula:



wherein B represents a peptide sequence, and R' represents a N(CH<sub>2</sub>-)<sub>2</sub>, NHCH<, or NHCH(CH<sub>2</sub>-)<sub>2</sub> group.

86. (Previously presented) The method according to claim 84, wherein the achiral acid is imino diacetic acid.

87. (Currently amended) The method according to claim 84 91, wherein the peptide sequences are derived from OspC protein of *Borrelia burgdorferi*.

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88. (Currently amended) The method according to claim 84 91 for preparing an LPA for presenting two identical C-terminal sequences Pro-Lys-Lys-Pro (Seq. ID 7) of OspC.

89. (Currently amended) The method according to claim 84 91, wherein the peptide sequences are derived from the flagellum of *Borrelia burgdorferi*.

90. (Currently amended) The method according to claim 84 91 for preparing an LPA selected from the group consisting of

[LPA-I] : FmocN(CH<sub>2</sub>CO-ProValValAlaGluSerProLysLysPro-OH)<sub>2</sub>,  
(FmocN(CH<sub>2</sub>CO-Seq. ID 1-OH)<sub>2</sub>)

[LPA-III] : NH<sub>2</sub>CH(CH<sub>2</sub>CO-ProValValAlaGluSerProLysLysPro-OH)<sub>2</sub>,  
(NH<sub>2</sub>CH(CH<sub>2</sub>CO-Seq. ID 1-OH)<sub>2</sub>)

[LPA-VII] : CH<sub>2</sub>(CH<sub>2</sub>CO-β-Ala-β-AlaLysGluProAsnLysGlyValAsnProAspGluValβ-Ala)<sub>2</sub>, (CH<sub>2</sub>(CH<sub>2</sub>CO-β-Ala-β-Ala-Seq. ID 4-β-Ala-OH)<sub>2</sub>)<sub>2</sub>

[LPA-VIII] : H<sub>2</sub>C(CH<sub>2</sub>CO-LysGluProAsnLysGlyValAsnProAspGluValβAla)<sub>2</sub>COOH (H<sub>2</sub>C(CH<sub>2</sub>CO-Seq. ID 4-β-Ala)<sub>2</sub>COOH),

[LPA-IX] : Fmoc-NHCH(CH<sub>2</sub>CO-AspArgValTyrIleHisProPheHisLeu-NH<sub>2</sub>)<sub>2</sub>,  
(Fmoc-NHCH(CH<sub>2</sub>CO-Seq. ID 5-NH<sub>2</sub>)<sub>2</sub>),

[LPA-X] : Alloc-NHCH(CH<sub>2</sub>CO-AspArgValTyrIleHisProPheHisLeu-NH<sub>2</sub>)<sub>2</sub>,  
(Alloc-NHCH(CH<sub>2</sub>CO-Seq. ID 5-NH<sub>2</sub>)<sub>2</sub>), and

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91. (Previously presented) A method of solid phase peptide synthesis for preparing a ligand presenting assembly (LPA) for presentation of at least two identical peptide sequences from *Borrelia burgdorferi* having between 4 and 20 amino acids and having free C-terminal groups comprising the steps of:

a) assembling a plurality of identical, fully side-chain protected peptide sequences on a single solid phase resin support to provide a compound having the following formula:



wherein S represents the solid phase resin support, A represents a peptide sequence having between 4 and 20 naturally occurring L-amino acid residues, and a is  $\geq 2$ , and represents the number of fully side-chain protected peptide sequences on the resin support;

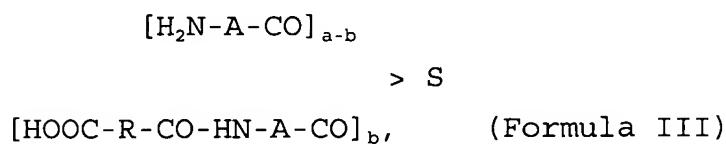
b) deprotecting any protected N-terminal amino groups while the peptide sequences are still attached to the resin support;

c) reacting the resulting compound having unprotected N-terminal amino groups in the peptide sequences with between 0.4 and 0.6 equivalents of an achiral dicarboxylic acid selected from the group consisting of: imino diacetic acid, 2-amino malonic acid, malonic acid, 3-amino glutaric acid and glutaric acid, being Fmoc, Boc or Aloc-protected on the amino or imino group, if present, thus having the following formula:



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wherein R represents a  $N(X)(CH_2)_2$ ,  $NH(X)CH<$ ,  $CH_2<$ ,  $NH(X)CH(CH_2)_2$  or  $CH_2(CH_2)_2$  group, and X represents an Fmoc, Boc or Aloc group, so that between 0.4 and 0.6 equivalents of said achiral dicarboxylic acid are added for every 1 equivalent of unprotected N-terminal amino group resulting in a compound with the following formula:



wherein b is between about 0.4a and 0.6a;

d) activating the product of step (c) (Formula III) so that the free carboxylic acid group reacts with the free N-terminal amino group; resulting in a compound of the following formula:



and

e) optionally splitting of any N-terminal Fmoc-group, Boc-group or Aloc-group;

f) cleaving the product of step (e) from the resin support resulting in a LPA peptide sequence having the following formula:

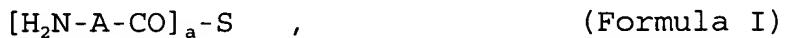


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wherein, if N is present in R, X represents H, an Fmoc, Boc or Aloc group, and Y is OH or NH<sub>2</sub>.

92. (Previously presented) A method of solid phase peptide synthesis for preparing a ligand presenting assembly (LPA) for presentation of at least two identical peptide sequences derived from OspC protein of *Borrelia burgdorferi* having between 4 and 20 amino acids and having free C-terminal groups comprising the steps of:

a) assembling a plurality of identical, fully side-chain protected peptide sequences on a single solid phase resin support to provide a compound having the following formula:



wherein S represents the solid phase resin support, A represents a peptide sequence having between 4 and 20 naturally occurring L-amino acid residues, and a is  $\geq 2$ , and represents the number of fully side-chain protected peptide sequences on the resin support;

b) deprotecting any protected N-terminal amino groups while the peptide sequences are still attached to the resin support;

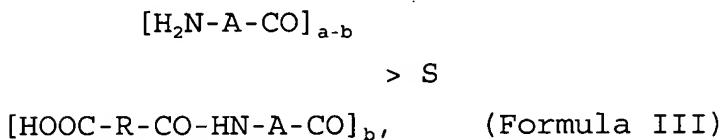
c) reacting the resulting compound having unprotected N-terminal amino groups in the peptide sequences with between 0.4 and 0.6 equivalents of an achiral dicarboxylic acid selected from the group consisting of: imino diacetic acid, 2-amino malonic acid, malonic acid, 3-amino glutaric acid and glutaric acid, being

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Fmoc, Boc or Aloc-protected on the amino or imino group, if present, thus having the following formula:



wherein R represents a  $N(X)(CH_2-)_2$ ,  $NH(X)CH<$ ,  $CH_2<$ ,  $NH(X)CH(CH_2-)_2$  or  $CH_2(CH_2-)_2$  group, and X represents an Fmoc, Boc or Aloc group, so that between 0.4 and 0.6 equivalents of said achiral dicarboxylic acid are added for every 1 equivalent of unprotected N-terminal amino group resulting in a compound with the following formula:



wherein b is between about 0.4a and 0.6a;

d) activating the product of step (c) (Formula III) so that the free carboxylic acid group reacts with the free N-terminal amino group; resulting in a compound of the following formula:

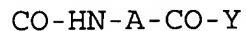


and

e) optionally splitting of any N-terminal Fmoc-group, Boc-group or Aloc-group;

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f) cleaving the product of step (e) from the resin support resulting in a LPA peptide sequence having the following formula:



R<



wherein, if N is present in R, X represents H, an Fmoc, Boc or Aloc group, and Y is OH or NH<sub>2</sub>.

93. (Previously presented) A method of solid phase peptide synthesis for preparing a ligand presenting assembly (LPA) for presentation of at least two identical peptide sequences from the flagellum of *Borrelia burgdorferi* having between 4 and 20 amino acids and having free C-terminal groups comprising the steps of:

a) assembling a plurality of identical, fully side-chain protected peptide sequences on a single solid phase resin support to provide a compound having the following formula:



wherein S represents the solid phase resin support, A represents a peptide sequence having between 4 and 20 naturally occurring L-amino acid residues, and a is  $\geq 2$ , and represents the number of fully side-chain protected peptide sequences on the resin support;

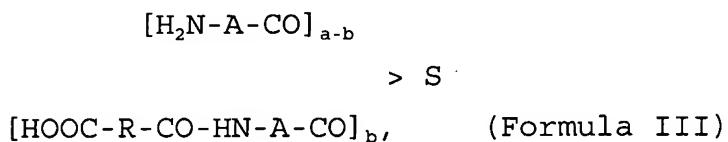
b) deprotecting any protected N-terminal amino groups while the peptide sequences are still attached to the resin support;

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c) reacting the resulting compound having unprotected N-terminal amino groups in the peptide sequences with between 0.4 and 0.6 equivalents of an achiral dicarboxylic acid selected from the group consisting of: imino diacetic acid, 2-amino malonic acid, malonic acid, 3-amino glutaric acid and glutaric acid, being Fmoc, Boc or Aloc-protected on the amino or imino group, if present, thus having the following formula:



wherein R represents a  $N(X)(CH_2-)_2$ ,  $NH(X)CH<$ ,  $CH_2<$ ,  $NH(X)CH(CH_2-)_2$  or  $CH_2(CH_2-)_2$  group, and X represents an Fmoc, Boc or Aloc group, so that between 0.4 and 0.6 equivalents of said achiral dicarboxylic acid are added for every 1 equivalent of unprotected N-terminal amino group resulting in a compound with the following formula:



wherein b is between about 0.4a and 0.6a;

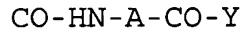
d) activating the product of step (c) (Formula III) so that the free carboxylic acid group reacts with the free N-terminal amino group; resulting in a compound of the following formula:



and

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- e) optionally splitting of any N-terminal Fmoc-group, Boc-group or Aloc-group;
- f) cleaving the product of step (e) from the resin support resulting in a LPA peptide sequence having the following formula:



R<



wherein, if N is present in R, X represents H, an Fmoc, Boc or Aloc group, and Y is OH or NH<sub>2</sub>.

94. (Withdrawn) A ligand presenting assembly (LPA) having a formula selected from the group consisting of



(1) HN<



(2) H<sub>2</sub>N-HC<



(3) H<sub>2</sub>C<



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CO-NH-A-CO-Y

(4)            H<sub>2</sub>N-CH<

CO-NH-A-CO-Y

or

CO-NH-A-CO-Y

(5)            CH<sub>2</sub><

CO-NH-A-CO-Y

obtained by the method of claim 84, wherein A represents a peptide sequence having between 4 and 20 naturally occurring amino acid residues, and wherein Y represents OH or NH<sub>2</sub>.

95. (Withdrawn) The method according to claim 85 for preparing an LPA selected from the group consisting of

[LPA-IV] : H-Lys-NHCH(CH<sub>2</sub>CO-ProValValAlaGluSerProLysLysPro-OH)<sub>2</sub>,  
(H-Lys-HNCH(CH<sub>2</sub>CO-Seq. ID 1-OH)<sub>2</sub>,

[LPA-XI] : Fmoc-AspProThrGlnAsnIleProProGly-NHCH(CH<sub>2</sub>CO-AspArgValTyrIleHisProPheHisLeu-NH<sub>2</sub>)<sub>2</sub> (Fmoc-Seq. ID 6-NHCH(CH<sub>2</sub>CO-Seq. ID 5-NH<sub>2</sub>)<sub>2</sub>).